Glucobrassicin, the Precursor of 3-Indolylacetonitrile, Ascorbigen, and SCN^- in *Brassica oleracea* Species

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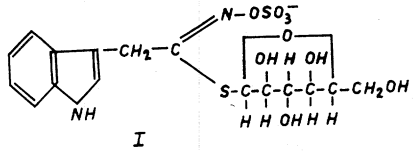
Recently we reported on the isolation of glucobrassicin[1], a new mustard oil glucoside occurring in fresh cabbage plants, which as a precursor of ascorbigen and SCN^- is of special biological interest. A tentative structure for glucobrassicin, based on the formation of ascorbigen, was suggested in the preliminary paper. Further results obtained by us have, however, made the ascorbigen structure proposed by Procházka *et al.*[2] improbable and lead us to propose the following formula for glucobrassicin (I).



I

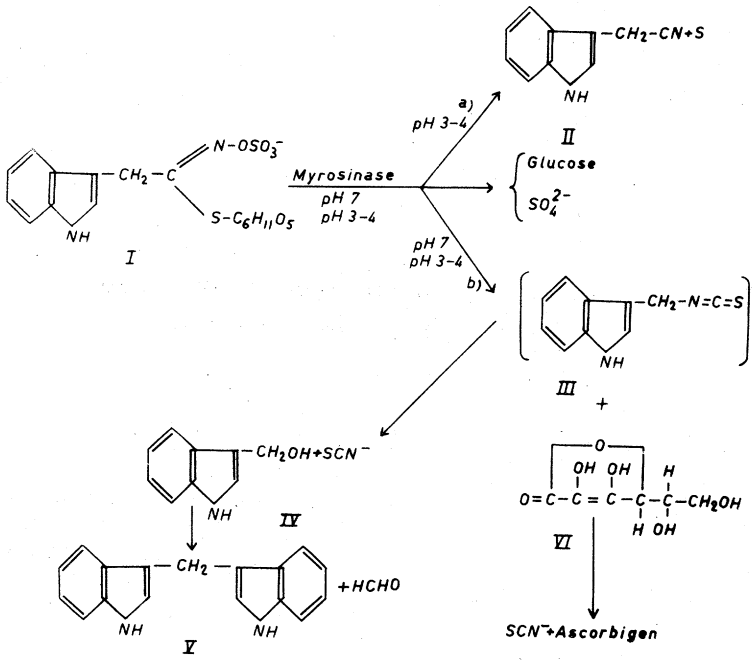
3-Indolylacet-thio(S-β-glucopyranosido)-hydroximyl-O-sulphate

Glucobrassicin (I) crystallizes as its tetramethylammonium salt in beautiful, rectangular plates melting at 140—142°C (Corr.) and containing one mole of crystal water. (Found: H_2O 3.29. Calc. for I: H_2O 3.45). The composition of the anhydrous substance is according to elementary analysis $C_{20}H_{31}N_3O_9S_2$ (521.6). (Found: C 46.20; H 6.28; N 7.91; O 27.42; S 11.69. Calc. for I: C 46.05; H 6.00; N 8.06; O 27.61; S 12.30).

The following findings support the above formula for glucobrassicin. Treatment of glucobrassicin with strong acids gives rise to hydroxylamine (detection by paper chromatography and colour reactions[3]), and enzymatic hydrolysis with myrosinase yields glucose and sulphate; both reactions are known to be typical for mustard oil glucosides. The for-

mation of an *iso*thiocyanate ester could not be detected; instead SCN^- was formed as mentioned in the previous paper.

Treatment of glucobrassicin in buffered solution at pH 3—4 with myrosinase yields 3-indolylacetonitrile and sulphur. Mustard oil glucosides are known to form nitriles under corresponding conditions[4]. The simultaneous formation of 3,3'-di-indolylmethane (detection by »Dünnschicht-Chromatographie»[5]), SCN^-, and formaldehyde (identification as the dimedone derivative by m.p.) can be understood if one assumes two different pathways (a) and (b) for the decomposition of glucobrassicin. In reaction (a) 3-indolylacetonitrile (II) is formed, and in reaction (b) SCN^- is split off from the hypothetical intermediate, 3-indolylmethyl-*iso*thiocyanate (III). 3-Hydroxymethylindole (IV) is formed from the indole part of the molecule and from this 3,3-di-indolylmethane (V) and formaldehyde. (The formation of 3,3'-di-indolylmethane and formaldehyde from 3-hydroxymethylindole has been shown by Thesing[6]).



Principal pathways of the splitting of glucobrassicin.

Like other mustard oil glucosides, glucobrassicin is hydrolyzed by hot acids and alkalies. The reactions proceed similarly as the enzymatic cleavage at pH 3—4. 3,3'-Di-indolylmethane was isolated from the alkaline hydrolysate in crude form (m.p. 160°C) and identified by comparison with a synthetic sample by »Dünnschicht-Chromatographie». Depending on conditions and reaction time, 3-indolylacetonitrile, 3-indolylacetamide, or 3-indolylacetic acid was identified by paper chromatography.

Treatment of an aqueous solution of glucobrassicin with Raney nickel[3] at room temperature gives skatole and tryptamine. Skatole was identified by »Dünnschicht-Chromatographie» and tryptamine by paper chromatography.

At pH 7 myrosinase splits off SCN^- quantitatively from glucobrassicin. This indicates that the *cleavage occurs only by pathway(b)*. 3-Hydroxymethylindole, which is unstable under the experimental conditions, is obviously formed from the indole part of the glucobrassicin

molecule because 3,3'-di-indolylmethane and a number of yet unidentified indole derivatives were observed.

Thiocyanate ion and ascorbigen were formed in the presence of ascorbic acid[1] (VI) during the enzymatic cleavage of glucobrassicin at pH 7. The reaction is practically quantitative if a sufficient amount of ascorbic acid is present, and also seems to occur via 3-hydroxymethylindole. This view is supported by a simple synthesis of ascorbigen by heating indole, formaldehyde, and ascorbic acid, or alternatively by heating 3-hydroxymethylindole and ascorbic acid in water: the synthetic products, authentic ascorbigen, and the ascorbigen formed enzymatically from glucobrassicin and ascorbic acid gave identical UV-spectra and behaved identically on paper chromatograms. The IR-spectra were identical except for minor differences in the range between 5.8 μ and 7.2 μ due to impurities. This strongly supports the view that the indole part in ascorbigen is linked by the methylene group to ascorbic acid. A detailed re-examination of the structure of ascorbigen is desirable.

The above facts show glucobrassicin to be a precursor of various indole compounds, some of which are growth factors. 3-Indoleacetonitrile, which was isolated by Henbest *et al.*[7] from cabbage, is a derivative of glucobrassicin. Also 3-indolylaldehyde and 3-indolylcarboxylic acid[8,9], which were found earlier in cabbage, can easily be derived from glucobrassicin.

Glucobrassicin, the first isolated mustard oil glucoside to contain a heterocyclic group, has chemical and physiological properties which the numerous previously known mustard oil glucosides do not have. Its abnormal behaviour — especially in the enzymatic cleavage — can be explained by mesomerism of its 3-indolylmethyl residue.

The existence of glucobrassicin has been predicted earlier[10], and a knowledge of its properties may help us to understand the origin and nature of various indole derivatives and growth-promoting substances found by various authors[7,11,12] in *Brassica oleracea* species. As a potential source of SCN^-, glucobrassicin may be responsible for the long known goitrogenic properties of cabbage. The investigations begun some years ago in this laboratory on plant substances which may disturb the function of the thyroid gland and possibly may be transferred from fodder to milk have, in fact, directed our attention to the factors in *Brassica* species[13, 14, 15, 16]. Glucobrassicin is one of these factors.

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